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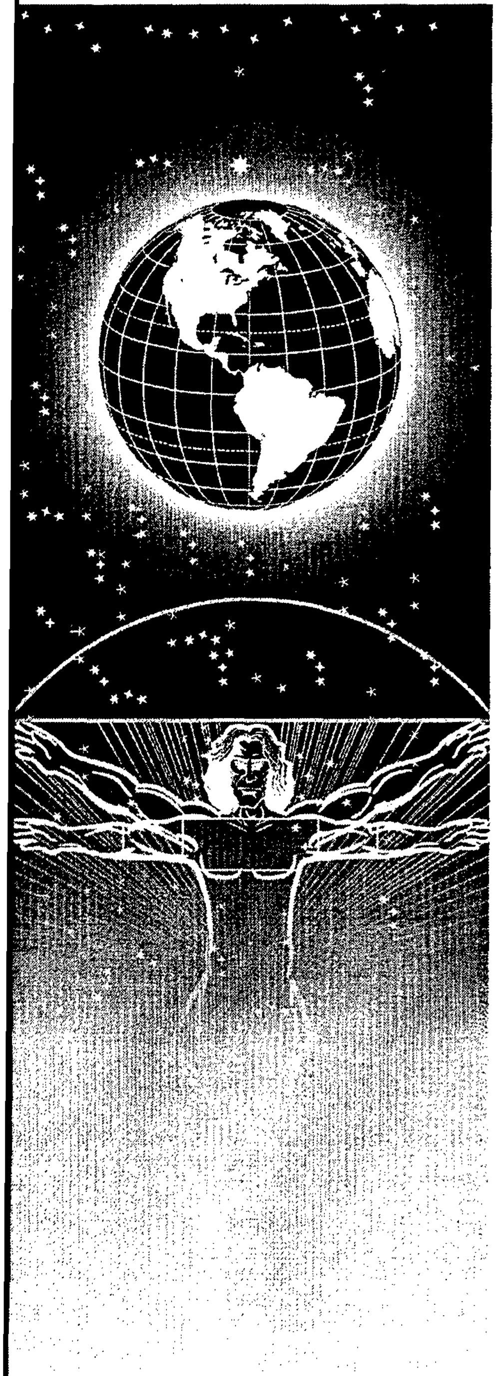

UNITED STATES AIR FORCE RESEARCH LABORATORY

REPRODUCTIVE EFFECTS OF JP-8 JET FUEL ON MALE AND FEMALE SPRAGUE-DAWLEY RATS AFTER EXPOSURE BY ORAL GAVAGE

D.R. Mattie
G.B. Marit
J.R. Cooper
OPERATIONAL TOXICOLOGY BRANCH, AFRL/HEST
2856 G STREET
WRIGHT PATTERSON AFB, OH 45433-7400

T.R. Sterner
OPERATIONAL TECHNOLOGIES CORP.
1370 N FAIRFIELD ROAD, SUITE A
BEAVERCREEK, OH 45432

C.D. Flemming
MANTECH-GEO-CENTERS JOINT VENTURE
P.O. BOX 31009
DAYTON, OH 45437-0009

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Human Effectiveness Directorate
Deployment and Sustainment Division
Operational Toxicology Branch
2856 G Street
Wright-Patterson AFB OH 45433-7400

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TECHNICAL REVIEW AND APPROVAL

AFRL-HE-WP-TR-2000-0067

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

David R. Mattie

DAVID R. MATTIE, Ph.D.
Acting Branch Chief, Operational Toxicology Branch
Air Force Research Laboratory

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ManTech-Geo Centers Joint Venture
PO Box 31009
Dayton, OH 45437-0009

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13. ABSTRACT *(Maximum 200 words)*

The jet engine fuel designated JP-8 is the standardized fuel for the US military, which has resulted in exposure to both male and female personnel. Limited information is available on the reproductive effects of JP-8 jet fuel in humans and animals. In this report data are presented from two reproductive studies. In the first study male rats were given 0, 750, 1500 or 3000 mg/kg neat JP-8 daily by gavage for 70 days prior to mating with naive females to assess fertility and sperm parameters. After 70 days of dosing, body weights in the 3000 mg/kg group were over 30% lower than control weights. There were no significant changes for pregnancy rate, gestation length or sperm parameters as compared to control values. In the second study female rats were dosed with neat JP-8 (0, 325, 750, 1500 mg/kg) daily by gavage for a total of 21 weeks (90-day plus mating with naive males, gestation and lactation) in an effort to assess general toxicity, fertility and reproductive endponts. Results of the general toxicity segment revealed a significant dose-dependent decrease in body weights of the female rats. Significant organ weight ratio increases were seen for the liver: body, liver:brain and kidney:brain weights. Corresponding histopathologic changes and increase in liver enzymes (ALT, AST) were not observed although there was an increase in liver weight. Significant pathological changes were limited to squamous hyperplasia of the stomach and perianal dermatitis. In the reproductive and fertility segment, there were no statistically significant changes from control values for gestation length, pregnancy rate and number of pups per litter. There was a trend for decreased pup weight with increasing dose from postnatal days 4 through 21 with the 1500 mg/kg pups statistically and biologically significantly lower on these days.

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PREFACE

This study was conducted at the Air Force Research Laboratory, Human Effectiveness Directorate, Operational Toxicology Branch (AFRL/HEST). Work was performed under the following contracts and supervision: ManTech GEOCENTERS Joint Venture (F41624-96-C-9010, Program Manager: Dr. Darol Dodd, P.O. Box 31009, Dayton, Ohio 45437) and Operational Technologies Corporation (DAHA 90-06-D-0014, Manager: Dr. Peter Lurker, 1370 N. Fairfield Rd., Suite A, Beavercreek, Ohio 45432).

The animals used in this study were handled in accordance with the principles in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, DHHS, National Institute of Health Publication #86-23, 1985 and the Animal Welfare Act of 1966, as amended.

Special thanks go to the animal care staff of AFRL/HEST led by Brenda Schimmel and Tim Bausman, and to pathology support, Peggy Parish of ManTech GEOCENTERS Joint Venture. The authors would also like to acknowledge Marcia Feldmann, also of ManTech GEOCENTERS Joint Venture, for painstakingly translating the lab results into electronic format and Chuck Goodyear for performing statistical analyses of the reproductive data. Mr. Goodyear is a statistical consultant for AFRL, Human Effectiveness Directorate, Crew Systems Interface Division (AFRL/HEC), Wright-Patterson Air Force Base, Ohio.

LIST OF ACRONYMS

°C	degrees Celsius
µm	micrometer
ALH	amplitude of lateral head displacement
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
BW	body weight
g	gram
Hz	Hertz
kg	kilogram
LOAEL	lowest observed adverse effect level
m^3	cubic meter
mg	milligram
mL	milliliter
n	sample number
NOAEL	no observed adverse effect level
PND	post-natal day
SE	standard error
s	second

REPRODUCTIVE EFFECTS OF JP-8 JET FUEL ON MALE AND FEMALE SPRAGUE-DAWLEY RATS AFTER EXPOSURE BY ORAL GAVAGE

INTRODUCTION

The jet engine fuel designated JP-8 is the standardized fuel for the U.S. military. The opportunity for exposure to JP-8 exists within many Air Force career fields. Skin and respiratory irritation symptoms have been reported in Air Force workers. The database for JP-8 toxicity is not complete. Limited information is available on the reproductive effects of JP-8 jet fuels in humans and animals.

A subchronic inhalation toxicity test of JP-8 was conducted by exposing male and female F-344 rats and C57BL/6 mice to JP-8 vapors at 0, 500 and 1,000 mg/m^3 on a continuous basis for 90 days, followed by recovery until approximately 24 months of age. Evaluation of data revealed limited toxicity and no tumor formation. The toxicity seen was either not a direct treatment effect of JP-8 or was due to the male rat specific alpha 2-microglobulin protein droplet nephropathy[1]. It is now recognized that the nephropathy seen in male rats is not expected to occur in humans[2]. Therefore, the limited toxicity seen in the JP-8 repeated dose study was not relevant to humans.

Clinical and histopathology parameters for the male rats in this study were previously reported by Mattie *et al*[3]. Male Sprague-Dawley rats were dosed with neat JP-8 (0, 750, 1500, 3000 mg/kg) daily by gavage for 90 days. Results revealed a significant dose-dependent decrease in body weights of rats exposed to JP-8. The male rat-specific alpha 2-microglobulin nephropathy was observed by histopathologic examination. A number of significant changes were also seen in blood and urine that were not dose-dependent. Additional treatment-related effects were gastritis and perianal dermatitis. Although there were no histopathological or weight changes in the livers of exposed rats, there were increases in the liver enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase). The elevated enzymes did not increase with increasing dose of JP-8[3].

A developmental toxicity study indicated that JP-8 is not a teratogen in the rat. Female rats were dosed with 0, 500, 1000, 1500 or 2000 mg/kg neat JP-8 daily by gavage on days 6 through 15 of gestation. Maternal body weights were significantly decreased in the 1000, 1500 and 2000 mg/kg/day dose groups while fetal weights were decreased in the 1500 and 2000 mg/kg/day groups. Fetal malformations and variations did not differ significantly between control and treatment groups[4].

In the first of two reproductive studies presented in this report, male rats were given 0, 750, 1500 or 3000 mg/kg neat JP-8 daily by gavage for 70 days prior to mating with naïve females to assess fertility and sperm parameters. In the second study, female rats were dosed with JP-8 (0, 325, 750 or 1500 mg/kg) daily by gavage for a total of 21 weeks (90-days plus mating with naïve males, gestation and lactation) in an effort to assess general toxicity, fertility and reproductive endpoints.

METHODS

Sprague-Dawley rats (Charles River Breeding Labs, Kingston, NY) were housed in plastic cages with Beta-Chip Hardwood Laboratory Bedding (Northeastern Products Corp., Warrensburg, NY). All rats, weighing 180 to 220 g upon receipt, were quarantined for two weeks prior to exposure. Feed (Formula 5008, Ralston Purina, St. Louis, MO) and water were available *ad libitum*. Ambient temperatures were maintained at 21-25°C with a light/dark cycle set at 12-hour intervals. Body weights were measured daily prior to dosing. Animals were examined daily for clinical signs of toxicity.

The JP-8 jet fuel was supplied by the U.S. Air Force (AFRL Propulsion Directorate, Wright-Patterson AFB, OH). The fuel met the requirements of Military Specification MIL-T-83133A. JP-8 was administered by gavage without a vehicle (neat). Control animals were dosed with 1.0 mL distilled water under the same conditions as test groups. Volumes to be administered each day were calculated from the individual daily body weights and the density of the test material (0.81 g/mL).

Male 90-Day Fertility Study

Young male Sprague-Dawley rats weighing 180-220 g were randomly assigned to 4 exposure groups. Each group contained a minimum of 20 male rats. The rats were given 0 (control), 750, 1500 or 3000 mg/kg JP-8 daily by gavage for 70 days prior to mating with naïve females. Male rats were cohabitated with one female at a time. In order to stagger delivery dates, male rats were paired with more than one female rat between 70 and 90 days of dosing with JP-8. Male rats were gavaged during cohabitation and returned to individual cages after successful mating. Exposure was continued until the rats were euthanized by carbon dioxide overdose at 90 days. General toxicity (clinical pathology, hematology and urinalysis) and histopathological effects of 90 day oral JP-8 exposure to male Sprague-Dawley rats were reported by Mattie *et al.* in 1995[3].

Fertility Analysis

Unexposed females mated with dosed males were allowed to give birth in order to determine gestation length. Successful mating (gestation day 0) was determined by presence of copulatory plug or sperm in a vaginal contents smear. Pregnancy rate (%) and gestation duration (days) were recorded for all dams. All rats were euthanized by carbon dioxide overdose.

Sperm Analysis

The epididymides were collected from each male rat at necropsy. The epididymides were then minced in phosphate buffered saline with bovine serum albumin; the resulting sperm suspension was videotaped in a Petroff Hauserr chamber. Determinations were made from the videotape using the CellSoft Automated Semen Analyzer (CRYO Resources, Ltd., New York, NY). Motility parameters measured by the CellSoft Analyzer were: sperm concentration, motile sperm concentration, percent motility, velocity, linearity, maximum amplitude of lateral head displacement (ALH), mean ALH and beat/cross frequency. The CellSoft Analyzer also measured the following parameters: mean radius, number of circular cells, percent circular cells/motile cells and percent circular cells/all cells.

Female 21-Week Exposure

Young female Sprague-Dawley rats weighing 180-200 g were randomly assigned to 4 exposure groups. Groups contained a minimum of 35 female rats. The rats were given 0 (control), 325, 750 or 1500 mg/kg JP-8 daily by gavage for 21 weeks (90-days followed by cohabitation, gestation, delivery and lactation). The male rats, not exposed to JP-8, were housed 1:1 with treated female rats. Dams were euthanized one day after weaning (Day 22 of lactation); male rats were euthanized after pregnancy was confirmed. Litters were standardized to four male pups and four female pups on postnatal day (PND) 5. Standardized litters were used for neurobehavioral tests (data not shown here); all rats were euthanized by carbon dioxide overdose.

Fertility and Viability Measures

Gestation day 0 was determined by presence of copulatory plug or sperm in a vaginal contents smear. Pregnancy rate (%) and gestation duration (days) were recorded for all dams. Size of the entire litter and the number born dead were noted on PND 1; the resulting numbers were compared between treatment groups. Pups were weighed on PND 1, 4, 7, 14, 21 and 90; male and female pup weights were compared between treatment groups and between sexes.

Clinical Pathology and Urinalysis

A subset of dams from each treatment group (maximum n of 10) was selected for hematology, clinical chemistry and urine analyses. The same subsets were also used for organ weights and histopathology. Whole blood was collected from the dam's inferior vena cava at necropsy. The following hematology parameters were measured: red blood cell count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width, mean corpuscular hemoglobin concentration, hematocrit, platelet count and differential leukocyte count. Determinations were made using an automated counter (H-1 System, Technicon Instruments, Corporation, Tarrytown, NY).

The following clinical chemistry parameters were measured in serum from dams: sodium, glucose, magnesium, carbon dioxide, potassium, albumin, chloride, total protein, calcium, blood urea nitrogen, total bilirubin, uric acid, inorganic phosphate, creatinine, triglycerides, cholesterol, AST, ALT, alkaline phosphatase, lactate dehydrogenase, creatine kinase and gamma-glutamyl transferase. Assays were performed with the Ektachem 700XR chemistry analyzer (Eastman Kodak, Rochester, NY).

The dams were transferred to metabolism cages upon weaning and urine was collected for 24-hours prior to sacrifice. The total volume of urine was noted and the urine was assayed for pH, specific gravity, total protein and creatinine. Specific gravity was measured with a refractometer (American Optical Corp., Southbridge, MA) and pH was measured with an electronic meter (Model 601A, Orion Research Inc., Cambridge, MA). Urine protein assays were performed on an automated chemistry analyzer (Model ACA IV, DuPont, Wilmington, DE). Urine creatinine determinations were made using the Ektachem 700XR analyzer.

Pathology and Organ Weights

A gross pathologic examination was performed on a subset of dams from each treatment group following euthanasia. A maximum of 10 rats per group was designated for clinical pathology and histopathology. The tissues listed in Table 1 were collected and prepared for histopathologic examination. Brain, kidneys, liver, spleen and ovaries were weighed during necropsy.

TABLE 1. TISSUES HARVESTED FROM CONTROL AND EXPOSED FEMALE RATS FOR HISTOPATHOLOGIC EXAMINATION

gross lesions	thymus
brain	kidneys
lungs	adrenals
trachea	pancreas
heart	ovaries, uterus
liver	nasal turbinates
spleen	esophagus
duodenum	stomach
jejunum	colon
ileum	rectum
urinary bladder	sternum
mandibular lymph nodes	sciatic nerve
mesenteric lymph nodes	skeletal muscle

Statistical Analyses

Statistical Analyses for General Toxicity Data

Adult body weights, organ weights and urine metabolites were analyzed by an ANOVA with multiple comparisons[7]. Clinical chemistry results, hematology values, urinalysis data and severity of pathological changes were compared using an ANOVA. The level of significance was accepted at $p \leq 0.05$ unless stated otherwise.

Statistical Analyses for Reproductive Measures

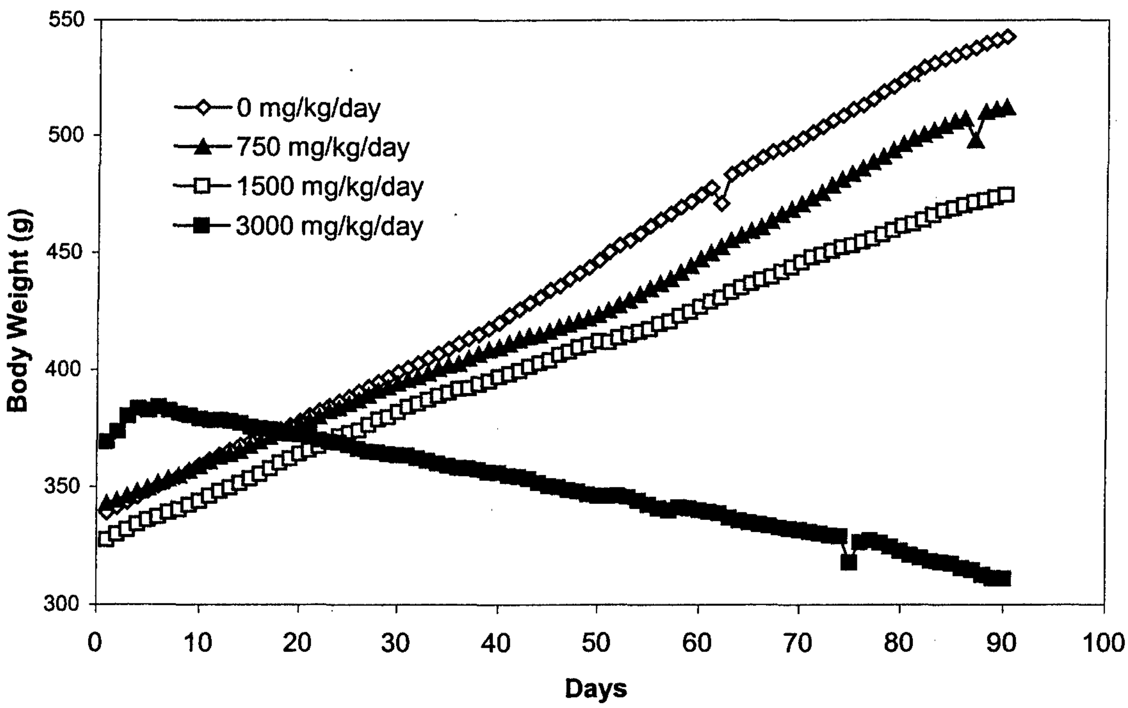
A one-factor (dose) or two-factor (dose and pup sex) analysis of variance (ANOVA) was performed for continuous variables. Error terms used were either dam(dose) or pup sex and dam(dose). One-way ANOVA was used with gestation lengths, sperm parameters and litter sizes while two-way was used for pup weights. Post-hoc paired comparisons of dose used two-tailed t-tests with pooled error.

For categorical variables, a Chi-square test of proportions was used to determine differences among the doses. Chi-square tests were used for pregnancy rates and percent viability. Post-hoc paired comparison for the viability parameter was performed with Fisher's Exact test. The level of significance was accepted at $p \leq 0.05$ unless stated otherwise.

RESULTS

Male 90-Day Fertility Study

There were no clinical signs of toxicity other than changes in body weight. Body weights in male rats decreased in a dose dependent manner during 90 days gavage exposure to JP-8 (Figure 1). A delay in starting dosing after randomly assigning rats to groups resulted in the 3000 mg/kg/day dose group being significantly heavier during the first weeks ($p \leq 0.05$). Body weights were not different between groups from days 9 through 25 of exposure. From day 26 through the end of the study, the 3000 mg/kg/day dose group was significantly lighter than the control group ($p \leq 0.05$). The 1500 mg/kg/day group body weights were significantly decreased on days 42 through 90 while the 750 mg/kg/day group weights were significantly lower on days 53 through 72 and days 81 through 90 ($p \leq 0.05$). Mortality was limited to one male rat in the 750 mg/kg/day dose group[3].



Source: Mattie *et al.*[3]

Figure 1. Mean Body Weights of Male Sprague-Dawley Rats Exposed by Gavage to 0, 750, 1500 and 3000 mg/kg/day JP-8 Jet Fuel for 90 Days

Gestation parameters for unexposed females mated with treated males are shown in Table 2. Pregnancy rates and gestation lengths were not adversely affected by paternal gavage exposure to JP-8. These parameters were not significantly different between dose groups. As a whole, these dams had low pregnancy rates, including the controls.

TABLE 2. GESTATION PARAMETERS OF UNEXPOSED DAMS MATED TO MALE RATS EXPOSED BY GAVAGE TO 0, 750, 1500 AND 3000 MG/KG/DAY JP-8 JET FUEL FOR 70 DAYS

Dose Group (mg/kg/day)	Number of Dams (n)	Pregnancy Rate (%)	Gestation Length (mean ± SE)
0 mg/kg/day	36	47	21.24 ± 0.26
750 mg/kg/day	38	39	21.07 ± 0.18
1500 mg/kg/day	42	57	21.08 ± 0.15
3000 mg/kg/day	32	53	21.41 ± 0.12

Epididymal sperm samples from males exposed by gavage to 0, 750, 1500 and 3000 mg/kg/day JP-8 for 90 days were evaluated using the CellSoft Automated Semen Analyzer. Table 3 contains sperm values for each dose group. The number of male rats per group ranged from 20 to 23. Outliers were removed after rigorous statistical analysis (Table 3) and were not related to dose. Significant differences were not found under any condition of analysis.

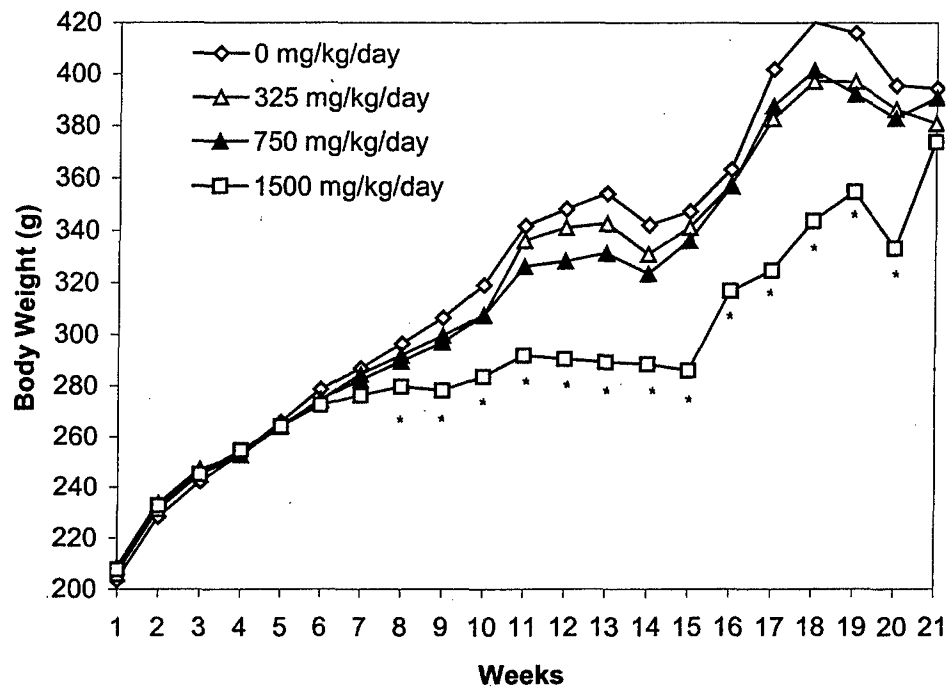
TABLE 3. SPERM PARAMETERS (MEAN ± SE) IN MALE RATS EXPOSED BY GAVAGE TO 0, 750, 1500 AND 3000 MG/KG/DAY JP-8 JET FUEL FOR 90 DAYS

Parameter	0 mg/kg/day	750 mg/kg/day	1500 mg/kg/day	3000 mg/kg/day
	(n=21)	(n=21)	(n=23)	(n=20)
Percent Motile	25.06 ± 2.07	29.60 ± 2.26	25.10 ± 1.59	24.60 ± 2.01
Concentration Motile (millions/mL)	0.21 ± 0.02	0.19 ± 0.02	0.20 ± 0.02	0.16 ± 0.02
Mean Velocity (μm/s)	112.21 ± 4.14	122.59 ± 5.64*	117.85 ± 5.09	117.04 ± 4.84
Mean Linearity	3.74 ± 0.14	3.70 ± 0.21	4.27 ± 0.16	4.04 ± 0.21
Max ALH (μm)	4.04 ± 0.19*	4.28 ± 0.24	4.06 ± 0.15	4.00 ± 0.19
Mean ALH (μm)	3.44 ± 0.15*	3.64 ± 0.18	3.47 ± 0.10	3.41 ± 0.14
Beat/Cross Frequency Hz (1/s)	10.42 ± 0.28	10.34 ± 0.23*	10.86 ± 0.19	10.70 ± 0.27
Average Radius (μm)	16.04 ± 1.19*	15.67 ± 0.92	15.93 ± 1.22*	13.79 ± 0.93
Circular % of Motile	14.18 ± 1.43	13.96 ± 1.72	17.26 ± 1.86	16.38 ± 2.3
Circular % of All Cells	3.87 ± 0.63	4.33 ± 0.71	4.23 ± 0.5	4.30 ± 0.72

Note: * One outlier removed

Female 21-Week Exposure

Results of the 21-week oral gavage exposure to 0, 325, 750 and 1500 mg/kg/day JP-8 revealed a decrease in body weights of the female rats (Figure 2). Body weights for the 1500 mg/kg/day rats were significantly lower than control rats ($p \leq 0.01$) starting at week 8 and continuing throughout gestation and most of lactation (weeks 13 through 20). Terminal body weights at week 21 were not significantly different from control rat weights. Mortality in each treatment group was not related to dose.



Note: * Significantly different at $p \leq 0.01$

Figure 2. Mean Body Weights of Female Sprague-Dawley Rats Exposed by Gavage to 0, 325, 750 and 1500 mg/kg/day JP-8 Jet Fuel for 21 Weeks (90 Days Plus Gestation and Lactation)

Gestation parameters for these females are shown in Table 4. Pregnancy rates and litter sizes for treated animals were not significantly different from controls. Gestation length was calculated from dams that became pregnant within one estrous cycle. Of the 87 dams that became pregnant, 77 took 1 to 4 days of cohabitation to become pregnant. The remaining 13 dams had reported times to impregnation ranging from 5 to 11 days. Most of these dams had gestation lengths as short as 14 days due to misidentification of the first day of impregnation. Therefore, time to impregnation plus gestation length was determined for each group. Since there were no significant differences between control and exposure groups for the combined impregnation/gestation length, dams with long impregnation times and short gestation lengths were excluded from the gestation length calculation. There were still no significant differences seen in gestation length for the remaining dams. The percentage of live pups on Day 1 for each dose group was not different from the control percent. The number of dams per treatment with at least one dead pup was not different between the dose groups and control (data not shown).

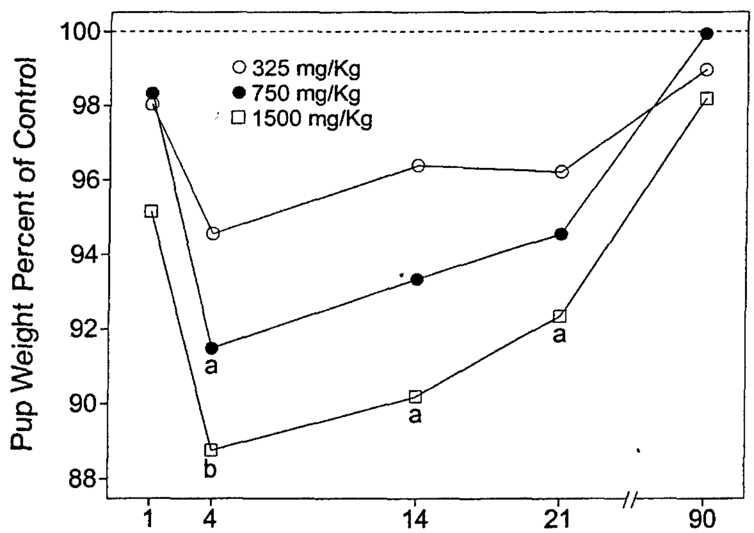
TABLE 4. GESTATION PARAMETERS OF EXPOSED DAMS MATED TO UNEXPOSED MALES - DAMS WERE EXPOSED BY GAVAGE TO 0, 325, 750 AND 1500 MG/KG/DAY JP-8 JET FUEL FOR 21 WEEKS

Dose Group (mg/kg/day)	Number of Dams (n)	Pregnancy Rate (%)	Gestation Length (Days: Mean ± SE)	Litter Size (Mean ± SE)	% Live Pups
0 mg/kg/day	41	73	22.00 ± 0.12	14.80 ± 0.71	97.3
325 mg/kg/day	37	59	21.85 ± 0.18	13.36 ± 1.02	97.3
750 mg/kg/day	35	63	21.83 ± 0.15	14.09 ± 0.99	99.4
1500 mg/kg/day	36	56	21.73 ± 0.41	14.45 ± 1.02	94.8

Pup weights were monitored on PND 1, 4, 14, 21 and 90. In Figure 3a, mean weights for each dose group are shown as percentages of the control weights. Pup data were included only from litters standardized on PND 4. A dose-related decrease in pup weights is evident. Pup weights (male and female) were significantly decreased in the 750 mg/kg/day dose group only on PND 4 ($p \leq 0.05$) (Figure 3a and 3b). The 1500 mg/kg/day dose group weights were significantly depressed from PND 4 ($p \leq 0.01$) through PND 14 and 21 ($p \leq 0.05$) but recovered by PND 90. The 1500 mg/kg/day pups were approximately 10% lower in weight than the controls from PND 4 through 21. Male pups were significantly heavier than female pups on all weighing days ($p \leq 0.05$).

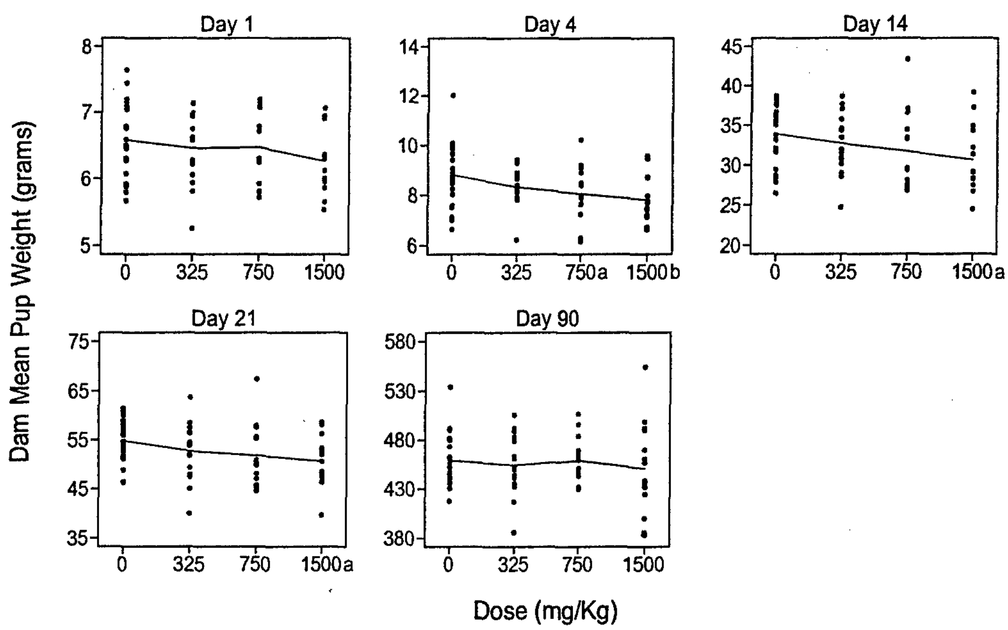
There was no difference in survival of pups between control and dose groups at PND 4, 14 and 21. Three pups, each from different litters in the 750 mg/kg/day dose group, did not survive between PND 21 and PND 90. Only one pup from the 1500 mg/kg/day group died during this time.

a



Notes: [a] $0.01<p\leq0.05$
[b] $0.001<p\leq0.01$

b



Notes: [a] $0.01<p\leq0.05$
[b] $0.001<p\leq0.01$

Figure 3. (a) Pup Body Weights as Percent of Control Weights. (b) Mean Pup Body Weight (g) for each Dam. Pups (male and female) from Female Sprague-Dawley Rats Exposed by Gavage to 0, 325, 750 and 1500 mg/kg/day JP-8 Jet Fuel for 21 Weeks (90 Days Plus Gestation and Lactation)

One day after weaning, the dams were euthanized. A subset from each group was necropsied. Due to sacrifices falling on weekends and the deaths of three animals not related to JP-8 dose (two from the 325 and one from the 750 mg/kg/day groups), the number of female rats per subset was limited. Treatment subsets had 7 or 8 rats while the control subset had 10 rats available. Organ weights are shown in Table 5. Liver weights were significantly increased, as were liver to body weight and liver to brain weight ratios ($p \leq 0.01$ in 1500 mg/kg/day group). Kidneys to brain ratios were also significantly increased ($p \leq 0.05$).

TABLE 5. ORGAN WEIGHTS AND RATIOS FOR FEMALE RATS EXPOSED BY GAVAGE TO 0, 325, 750 OR 1500 MG/KG JP-8 FOR 21 WEEKS

	Mean ± SE (g)			
	Control (n=10)	**325 mg/kg/day** (n=7)	**750 mg/kg/day** (n=8)	**1500 mg/kg/day** (n=8)
Body	351.0 ± 12.6	354.3 ± 9.0	357.5 ± 6.5	348.8 ± 16.3
Brain	1.96 ± 0.03	1.91 ± 0.04	1.97 ± 0.02	1.93 ± 0.05
Liver	14.8 ± 0.8	15.7 ± 0.6	**17.8 ± 0.7[a]**	**19.1 ± 0.7[b]**
Kidneys	2.9 ± 0.1	2.9 ± 0.1	3.2 ± 0.1	3.2 ± 0.1
Spleen	0.71 ± 0.02	0.65 ± 0.03	0.67 ± 0.04	0.66 ± 0.01
Brain:BW	0.56 ± 0.01	0.54 ± 0.02	0.55 ± 0.01	0.56 ± 0.03
Liver:BW	4.20 ± 0.1	4.4 ± 0.1	**5.0 ± 0.2[b]**	**5.5 ± 0.1[b]**
Kidneys:BW	0.83 ± 0.02	0.82 ± 0.02	0.90 ± 0.02	0.93 ± 0.05
Spleen:BW	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Liver:Brain	754.9 ± 38.9	823.6 ± 39.8	**903.1 ± 31.8[a]**	**993.6 ± 46.3[b]**
Kidneys:Brain	148.8 ± 3.82	152.3 ± 4.7	**163.6 ± 3.2[a]**	**165.4 ± 3.9[a]**
Spleen:Brain	36.4 ± 1.1	34.1 ± 1.5	34.3 ± 1.8	34.1 ± 1.1

Notes: BW = body weight
[a] Significantly different than control at $p<0.05$.
[b] Significantly different than control at $p<0.01$.

No significant changes were found in urine parameters (total volume, specific gravity and creatinine concentration). There were no statistically significant changes in most hematology counts: neutrophil, eosinophil, basophil, lymphocyte and platelet. The leukocyte count was found to be significantly decreased in the 325 mg/kg/day dose group alone ($p \leq 0.05$). This change was not dose dependent and has questionable biological significance. Clinical chemistry values (sodium, chloride, glucose, triglycerides, creatinine, alkaline phosphatase, AST, ALT) for treatment groups were not significantly different from control values. Data are not shown for urine, hematology and clinical chemistry parameters.

Samples of all collected tissues for each rat in the subsets were not available for histopathological examination. Significant pathological changes were limited to squamous hyperplasia of the stomach and perianal dermatitis. The incidence and severity of these changes were found to be dose-dependent and statistically significant at 1500 mg/kg/day for perianal dermatitis and stomach hyperplasia ($p \leq 0.05$, see Table 6). Only the incidence and severity of the squamous hyperplasia of the stomach were significantly increased after oral exposure to 750 mg/kg/day JP-8 ($p \leq 0.05$).

TABLE 6. INCIDENCE AND SEVERITY OF PERIANAL DERMATITIS AND STOMACH HYPERPLASIA IN FEMALE RATS EXPOSED BY GAVAGE TO 0, 325, 750 OR 1500 MG/KG JP-8 FOR 21 WEEKS

	Mean ± SE			
	Control	325 mg/kg/day	750 mg/kg/day	1500 mg/kg/day
ANUS	(n=8)	(n=4)	(n=5)	(n=6)
Dermatitis				
-Incidence	0	0	0	5
-Severity	0.0	0.0	0.0	**1.2 ± 0.3***
Hyperplasia				
-Incidence	0	0	0	3
-Severity	0.0	0.0	0.0	0.7 ± 0.4
STOMACH	(n=7)	(n=4)	(n=5)	(n=6)
Gastritis				
-Incidence	0	2	1	0
-Severity	0.0	0.5 ± 0.3	0.2 ± 0.2	0.0
Hyperplasia				
-Incidence	0	3	5	6
-Severity	0.0	0.8±0.3	**1.6±0.3***	**1.7±0.2***

Notes: * Significantly different than control at $p<0.05$.
Incidence = number of animals in which lesion occurred
Severity = mean of animals in a group (n) where: 0 = no change, 1 = minimum or very slight, 2 = slight degree, 3 = moderate or middle degree, 4 = marked and 5 = maximum

DISCUSSION

Male 90-Day Fertility Study

Clinical Signs

Except for body weight changes, daily exposure to JP-8 by gavage for 90 days resulted in no adverse clinical signs or mortality. The lowest exposure dose of 750 mg/kg caused a significant decrease in body weights ($p<0.05$). This is consistent with exposure to other jet fuels and other routes of exposure. Exposure to vapors of JP-8 as low as 500 mg/m^3 for 90 days significantly lowered the body weight of male and female rats[1]. The Navy uses JP-5, a similar mixture to JP-8 with a higher boiling point. Exposure to JP-5 at 150 mg/m^3 resulted in significantly lower body weights after 90 days of continuous exposure[5]. JP-4 jet fuel depressed body weights significantly in animals exposed for 90 days continuously to 500 mg/m^3 or to 1000 mg/m^3 for 12 months intermittently[6,7].

Sperm Analysis

There were no indications that oral exposure to JP-8 at doses as high as 3000 mg/kg for 90 days caused any effects on the sperm parameters measured and analyzed statistically. Briggs[8] exposed male rats for 6 hours/day, 5 days/week for 6 weeks to 1000 mg/m^3 JP-8 vapor (whole body exposure). After an 87 day recovery period, they evaluated sperm from the cauda. Sperm concentration and motility were not different between control and JP-8 exposed rats. Mean percent motility for control rats was 48%. Control motility values for both Briggs' study and this study were lower than normally seen in rat fertility studies[9]. Morphology was only observed visually by microscopic examination but appeared to be within normal limits[8]. Exposure to JP-8 appears to have no effect on sperm of male rats, either immediately after repeated exposure or following a recovery period.

LeMasters *et al.*[10] examined sperm quality in 50 men performing aircraft maintenance at an Air Force base. Sperm analyses (concentration, motility, viability, morphology, morphometry and stability of sperm chromatin) were performed at 15 and 30 weeks after exposure began. Exposures were only 10% of permissible exposure limits. No significant association between the sperm quality of maintenance workers and jet fuel exposure (primarily JP-4) was seen.

Fertility of Dams

Although pregnancy rates were low across control and dose groups, they were not related to exposure of the male rats to JP-8. Unexposed female rats in the two highest dose groups became pregnant at higher percentages than the control rats. Based on the results of this study, exposure of the male mating partner to JP-8 appears to have no effect on fertility of unexposed female rats.

Female 21-Week Exposure

Clinical Signs

Daily exposure to JP-8 by gavage for 90 days resulted in no adverse clinical signs or mortality. Body weights were decreased in all female rats dosed with 1500 mg/kg JP-8. This is consistent with other jet fuel exposures as described above[1,5-7]. The dose of JP-8 that resulted in decreased body weight of female rats was twice as high as the dose that decreased male rat weights in the current study.

Fertility and Viability Measures

JP-8 administration by gavage resulted in no statistically significant changes from control values for pregnancy rates, gestation lengths and numbers of pups per litter. Mean gestation length was essentially identical across groups. The average litter size from exposed dams was not affected by exposure to JP-8. There is no evidence that viability and survival of the pups was affected by exposure of their dams to JP-8.

Pup Weights

The decrease in maternal body weights in the 1500 mg/kg/day group was mirrored by a trend in decreased pup weights on PND 4 through PND 21. Although the pups were lighter across the treated groups in a dose dependent manner, only the 1500 mg/kg/day dose group pups were approximately 10% lower in weight than the control pups during this period. Decreased body weight is generally considered biologically significant at 10% or greater [11]. The 750 mg/kg/day pup weights were only significantly different at PND 4 ($p \leq 0.05$) and the decrease was less than 10%. All pups recovered to comparable control weights after weaning.

General Toxicity

General toxicological effects were limited to increased liver and relative liver weights in the 750 and 1500 mg/kg/day dose groups, as well as increased liver to brain and kidney to brain ratios in the same groups. Corresponding histopathologic changes and increases in liver enzymes (ALT, AST) were not observed. Although liver enzymes were elevated in a JP-8 oral dosing study using male rats, there was no increase in liver weight[3]. Liver weights were also not different between control and male and female exposed rats after inhalation exposure to vapors of JP-8 for 90-days[1]. After inhalation of aerosolized JP-8, liver weights were not significantly higher than control rats. However, relative liver weights were elevated in the 1000 mg/m^3 dose group in both the 7 and 28 day repeated dose exposures and in the 500 mg/m^3 dose group in the 28 day exposure[12]. Urine, hematology and serum chemistry values were not adversely affected by oral exposure to JP-8.

Pathology

Significant pathological changes ($p \leq 0.05$) were limited to squamous hyperplasia of the stomach and perianal dermatitis. Both changes are apparently the result of irritation of the squamous epithelium of the stomach due to oral dosing and to unabsorbed jet fuel coming in contact with the perianal epidermis during defecation. Perianal dermatitis and gastritis were reported after oral exposure of male rats to JP-8[3]. Baker *et al.*[13] exposed male Fischer 344 rats to JP-8 via dermal application once daily for 4 weeks. This repeated exposure to 0.156 ml/day JP-8 resulted in a thickened epidermis produced by hyperplasia, hyperkeratosis and dermatitis. In addition, degenerative changes such as ulceration were seen. All of these studies have shown that the stratified squamous epithelium of the skin in rats is irritated by repeated exposure to JP-8.

CONCLUSIONS

JP-8 was found to have limited reproductive and developmental toxicity in Sprague-Dawley rats. The no observed adverse effect levels (NOAELs) for fertility in male and female rats were the highest doses tested, 3000 and 1500 mg/kg/day, respectively. The lowest observed adverse effect level (LOAEL) for JP-8 developmental effects was 1500 mg/kg/day (maternal exposure) due to decreased pup body weights. Therefore, the NOAEL for pup weights was 750 mg/kg/day.

Oral exposure to JP-8 jet fuel was irritating to the squamous epithelium of the stomach and perianal region of female rats in the 750 and 1500 mg/kg/day dose groups. Liver and relative liver weights increased at the same doses. As a result, the JP-8 oral NOAEL in adult female rats was 325 mg/kg/day. Similarly, the LOAEL for adult males rats exposed to JP-8 orally was 750 mg/kg/day due to changes in clinical pathology, body weight, organ weights and the same irritation seen in female rats[3]. The NOAEL would be less than 750 mg/kg/day in male rats. Changes in male rats may be complicated by the male rat-specific nephropathy produced after exposure to hydrocarbon fuels such as JP-4 and JP-8[1,3,6,7].

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